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Urothelial differentiation in chronically urine-deprived bladders of patients with end-stage renal disease

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Background. It is unknown whether normal bladder voiding function, or soluble factors present in urine, contribute to the maturation and maintenance of the differentiated state of the uroepithelial cell lining of the lower urinary tract.

Methods. We used the urothelium of anuric patients on long-term hemodialysis, sampled at the time of renal transplantation, to investigate the expression of urothelial differentiation-associated antigens, including uroplakins (UPIa, UPIb, UPII, and UPIIIa), cytokeratin isotypes (CK7, CK8, CK13, CK14, CK17, CK18, and CK20), nuclear hormone receptors [peroxisome proliferators activated receptor-γ (PPAR-γ) and retinoid X receptor-α (RXR-α)], and a cell cycle marker (Ki-67). To determine whether urinary metabolites of the arachidonic pathway could induce urothelial differentiation, cultured normal human urothelial (NHU) cells were treated with 15-deoxy-Δ12, 14-prostaglandin J_2 (15d-PGJ_2) and prostaglandin J_2 (PGJ_2). The expression levels of the markers of differentiation, the uroplakins, were assessed by ribonuclease protection assay.

Results. When compared in a blinded analysis against control normal urothelium, no significant changes were found in the expression or localization patterns of any of the antigens studied in the anuric patients. Furthermore, neither 15d-PGJ_2 nor PGJ_2 were able to induce expression of the UPII gene in NHU cells, in contrast to cultures exposed to the pharmacologic PPAR-γ agonist, troglitazone.

Conclusion. These data provide prima facie evidence that exogenous urine-derived factors do not modulate the differentiation program in urothelium, suggesting that other urothelial- or serum-derived factors are likely to be involved. These findings are important in understanding postdevelopmental maturation and functional relationships in urologic tissues of the adult organism.

Human urothelium undergoes a complex differentiation process that culminates in the formation of asymmetric unit membrane plaques in the apical membrane of the terminally differentiated superficial cells. The asymmetric unit membrane, which is assembled by the interactions of at least four integral transmembrane uroplakin proteins, UPIa, UPIb, UPII, and UPIIIa, is regarded as an unequivocal marker of terminal urothelial differentiation [1]. The critical role of the asymmetric unit membrane in developing an effective urinary barrier is illustrated in the UPIIIa null mouse, which has a "leaky" urothelium associated with aberrant plaque formation [2, 3]. However, the in vivo factors that direct and maintain the normal urothelial differentiation program are poorly understood.

Using a well-characterized culture system for normal human urothelial (NHU) cells [4, 5], we have recently shown that synthetic high-affinity activators of peroxisome proliferators activated receptor-γ (PPAR-γ) induce a program of gene expression changes leading to the expression of markers of terminal urothelial differentiation, including cytokeratin (CK) 20 [6] and the uroplakins [7]. The PPAR-γ–induced differentiation was contingent upon coinhibition of an epidermal growth factor receptor (EGFR)-mediated autocrine signaling pathway, which is active in proliferating urothelial cells [7]. This implies a critical balance in the regulation of proliferation and differentiation in urothelium, an epithelium characterized by a low constitutive turnover rate, but high proliferative and regenerative capacity.

PPAR-γ belongs to the superfamily of nuclear hormone receptors and is a ligand-activated transcription factor that requires heterodimerization with the retinoid X receptor-α (RXR-α) to bind specific PPAR response elements (PPRE) in the promoter region of target genes [8, 9]. PPAR-γ expression has been described in the mature urothelium of mice, rabbits, and humans and has also been identified in the developing urothelium of the mouse urogenital sinus [10–12]. In mature urothelium, the most intense nuclear expression of PPAR-γ is present in the superficial cells [6, 13] a pattern that is obscured in conditions of urothelial squamous metaplasia [6].

15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2) has been identified as a candidate natural ligand for PPAR-γ

Key words: urothelium, PPAR, differentiation, anuria, prostaglandin, bladder.

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Table 1. Patient details

Case	Gender	Age	Anuria	Volume per day in mL (micturation frequency per 24 hours)	Dialysis *months*	Histology	Ki-67 index
1	M	22	Yes	—	77	Normal	0
2	F	37	Yes	—	19	Normal	0.1
3	M	28	Yes	—	12	Normal	0.2
4	M	57	Yes	—	17	Mild inflammation	0.2
5	F	61	Yes	—	79	Normal	0.1
6	M	27	Yes	—	56	Mild inflammation	0.04
7	M	37	No	350 (1)	34	Mild inflammation	0.2
8	M	40	No	300 (2)	30	Normal	0.1
9	F	58	No	50 (2)	26	Normal	0.1
10	M	37	No	500 (1)	19	Normal	0.1

and is the major prostaglandin present in urine [14]. This implies that superficially positioned urothelial cells are exposed to factors in urine that may be capable of activating the terminal urothelial differentiation program.

In this study we tested the hypothesis that factors in urine, in particular urine-derived 15d-PGJ_2 acting through PPAR-γ, are necessary for regulating and maintaining a terminally differentiated urothelial phenotype. For this purpose, bladder urothelia from patients on long-term hemodialysis with no normal urine production were compared to the urothelia from patients with normal renal function, with respect to histomorphology, proliferation (Ki-67 antigen) and the expression of antigens associated with urothelial differentiation, including uroplakins, CKs, and PPAR-γ. Finally, NHU cell cultures were exposed to 15d-PGJ_2 in the presence or absence of EGFR inhibitors, in order to determine whether 15d-PGJ_2 could activate the terminal urothelial differentiation program by inducing uroplakin gene expression [6, 7].

METHODS

Clinical specimens

The collection of all surgical specimens was approved by the relevant Local Research Ethics Committee. Tissues were fixed in 10% buffered formalin and processed into paraffin wax for histology or immunohistochemistry. Histopathologic assessment for each case was performed on hematoxylin and eosin–stained sections using standard criteria for the evaluation of urothelium [15].

Open bladder mucosal biopsies were obtained from ten consented patients (mean age 40 years; range 22 to 61 years) undergoing surgery for renal allograft transplantation between 1998 and 1999. The biopsies originated from the vesicoureteric anastomosis site at the superior-lateral aspect of the bladder. All patients had end-stage renal disease (ESRD) and had been on maintenance hemodialysis for a minimum of 12 months (mean dialysis time 37 months; range 12 to 79 months). All patients participating in the study answered questions about continued residual urine production (i.e., amount and frequency of diuresis per 24 hours) (Table 1). At the conclusion of the study, patients were directly contacted about urinary symptoms and in addition, patient medical history was reviewed regarding bladder function and any novel lower urinary tract pathology.

Nine bladder biopsies were selected at random from a bank of anonymous normal bladder controls. The specimens had been taken from patients with no history of renal function disorders and all had been classed as showing a normal, intact mature urothelium with no evidence of inflammation, ulceration, dysplasia, or malignancy by histopathologic criteria. The clinical reasons for cystoscopy were varied and included investigations for hematuria, repeated atypical cells in urine, a previous history of low grade papillary urothelial carcinoma, and pediatric patients undergoing reconstructive bladder surgery.

Immunohistochemistry

Immunohistochemistry was performed using rabbit antisera against UPIa (generously provided by T. Yoshiki, Department of Urology, Shiga University of Medical Science, Japan [16]), UPIb (the generous gift of W. Adachi, Department of Ophthamology, Kyoto Prefectural University of Medicine, Japan [17]) and monoclonal anti-UPIIIa (clone AU1) (purchased from Progen Biotechnik, Heidelberg, Germany). CK expression was localized using monoclonal antibodies to CK7 (clone LP1K), CK8 (clone LE41), CK14 (clone LL002), CK19 (clone LP2K) (all supplied by Central Resources, Cancer Research UK, London, UK), CK13 (clone IC7) (from ICN Biomedical Inc., Basingstoke, UK), CK17 (clone E3, supplied by Serotec, Kidlington, UK), CK18 (clone CY-90) (from Sigma-Aldrich, Poole, UK), and CK20 (clone Ks20.8) (supplied by Dako, Ely, UK). Monoclonal anti-PPAR-γ (clone E-8) and polyclonal anti-RXR-α (clone D20) were obtained from Santa Cruz Biotechnology (supplied by Autogen-Bioclear UK Ltd., Calne, UK), and

monoclonal anti-Ki-67 (clone MIB-1) was supplied by Dako. All antibodies were titrated prior to use.

Five micrometer sections were dewaxed in xylene and rehydrated through graded alcohols. Endogeneous peroxidase activity was inactivated in 3% (vol/vol) hydrogen peroxide for 10 minutes, followed by blocking of endogenous avidin-binding sites using an avidin/biotin kit (Vector Laboratories, Peterborough, UK), according to the manufacturer's protocol. Heat antigen retrieval, in which sections were boiled for 10 minutes in 10 mmol/L citric acid buffer, pH 6.0, in a microwave oven, was required prior to labeling sections with antibodies against CK7, CK8, CK18, UPIa, UPIb, UPIIIa, RXR-α, and MIB-1. For antibodies against CK13, CK14, CK17, and PPAR-γ, heat antigen retrieval was followed by an additional 1 minute of digestion of tissue sections in 0.1% (wt/vol) trypsin in 0.1% $CaCl_2$, pH 7.6, at 37°C. Antibodies for CK19 and CK20 required digestion for 10 minutes in trypsin in 10 mmol/L Tris-buffered saline, pH 7.6. Immunohistochemistry for all antibodies, except PPAR-γ and RXR-α, was performed using an indirect strepavidin ABC immunoperoxidase method (Dako), according to the manufacturer's recommendations. For the detection of low-density PPAR-γ and RXR-α antigens, the more sensitive tyramide-based catalyzed signal amplification method [Dako Catalysed Signal Amplification (CSA) peroxidase method] was applied. The manufacturer's recommendations were followed, except that in order to optimize the signal-to-noise ratio, the incubation in primary antibody was extended to 30 minutes and the secondary antibody incubation and amplification period were reduced to 5 minutes each. All labeling series were performed with appropriate negative and positive controls. Slides were counterstained with Mayer's hemotoxylin, dehydrated and mounted in DPX (BDH, supplied by Merck, Lutterworth, UK).

The specificity of the labeling characteristics of PPAR-γ achieved using the CSA Kit was assessed on mature adipose tissue and large bowel mucosa. In large bowel, the differentiated colonic epithelial cells facing the lumen displayed intense nuclear labeling, whereas proliferating cells located in the bases of the crypts labeled not at all, or very weakly (data not shown). These results were consistent with those described in mouse colonic mucosa [18].

Objective evaluation of expression patterns and parameters

High power images (×400 magnification) were selected from areas of intact, full-thickness urothelium and captured using a digital camera (JVC, KY-F55BE, Victor Company of Japan) mounted on a light microscope (Zeiss Axioplan 2; Carl Zeiss, Ltd., Welwyn Garden City, UK). A high-quality print of a representative captured field for all cases were ranked by two observers (J. Southgate and J. Stahlschmidt), blinded to their origin, for labeling patterns of UPIa, UPIb, UPIIIa, CK20, and PPAR-γ. The Kruskal-Wallis nonparametric test was used to analyze differences in the expression patterns between "anuric," "non-anuric," and "control" categories. Ranking was inferred from reference labeling patterns of normal urothelia.

The Ki-67–labeling index was assessed in areas of full-thickness urothelium by counting the proportion of nuclei with a positive (diffuse or punctate) Ki-67 nuclear labeling pattern [19]. The number of total nuclei assessed per biopsy ranged from 800 to 3000.

Cell culture

NHU cells were isolated and established in culture as finite cell lines, as previously detailed [4, 5]. Cells were maintained in keratinocyte serum-free medium (KSFM), containing bovine pituitary extract and EGF at the manufacturer's recommended concentrations (Invitrogen Ltd., Paisley, UK) and 30 ng/mL cholera toxin (Sigma-Aldrich, Poole, UK). NHU cell lines were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air and were used for these studies between passages 3 and 5.

In order to determine whether 15d-PGJ_2 or its metabolic precursor, PGJ_2, induce differentiation of NHU cells, cells were treated with 15d-PGJ_2 and PGJ_2. Experiments were performed using the optimized conditions described previously [6, 7]. Briefly, NHU cells were seeded at 4×10^4 cells/cm^2 and grown to 70% confluence, before being treated with 1 μmol/L to 5 μmol/L 15d-PGJ_2 (Calbiochem-Novabiochem Biosciences Ltd., Nottingham, UK), 0.5 μmol/L to 20 μmol/L PGJ_2 (Sigma-Aldrich, Poole, UK) or 0.1 μmol/L to 5 μmol/L troglitazone (a gift from Parke-Davis Pharmaceutical Research, Ann Arbor, MI, USA) as positive control, or 0.01% (vol/vol) dimethyl sulfoxide (DMSO) as negative (solvent) control. After 24 hours, the medium was removed and the cells were maintained with 1 μmol/L EGFR inhibitor, PD153035 (Calbiochem-Novabiochem Biosciences Ltd.), which was replenished with the medium every 2 days. Cell cultures were harvested and assessed for uroplakin transcripts at 4 days.

Ribonuclease protection assays

To extract RNA from cell monolayers, Trizol™ (Invitrogen) was added to the cell monolayer (1 mL/10 cm^2) and the cell lysate was scraped into a centrifuge tube. RNA was extracted as recommended by the manufacturer.

Part-length cDNA fragments of the coding region for human *UPIa, UPIb, UPII, UPIIIa*, and *GAPDH* genes were cloned into *pGEM-T Easy* (Promega, Southampton, UK), as described previously [20]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal riboprobe control. ^{32}P-labeled antisense

transcripts of the cDNAs of interest were generated from linearized plasmids using an In Vitro Transcription Kit (Promega), according to the manufacturer's protocol. After DNAse treatment, riboprobes were purified by passage through Chromaspin 30-DEPC columns (BD Biosciences Clontech UK, Oxford, UK).

Approximately 2 fmol of each riboprobe were mixed and hybridized to 5 μg of total RNA using an RPAIII Kit (Ambion Ltd., Huntingdon, Cambs, UK), according to the manufacturer's protocols. Products were separated on 5% denaturing polyacrylamide gels (Sequagel) (from Flowgen, Lichfield, UK), visualized by autoradiography and quantified by means of a phosphorimager (Bio-Rad GS-525 Molecular Imager System) (Hemel Hempstead, UK).

RESULTS

Histologic sections of "control," "anuric," and "nonanuric" bladder urothelia were similar and revealed the characteristic vertical organization pattern into three well-defined cell layers. The urothelial thickness was between four to seven cell layers in all categories. Superficial cells were the largest cells of either flattened or rounded shape, often binucleated with large nuclei and slightly open, sometimes vesicular chromatin and occasional large nucleoli. There was no evidence of metaplastic changes or atypia. Mitotic figures were scanty and observed in basal and intermediate cell layers. Occasional intraurothelial lymphocytes were present and the lamina propria contained occasional mononucleated inflammatory cells, including macrophages or mast cells. Two "anuric" (56 and 36 months on dialysis) and one "nonanuric" (17 months on dialysis) specimens showed mild chronic inflammation of the urothelium and lamina propria. No acute inflammatory cells, plasma cells, or granulomata were identified. One control bladder biopsy revealed von Brunn's nests.

Uroplakin expression

All control urothelia reacted with the three antiuroplakin antibodies (Fig. 1, left column). UPIa and UPIIIa showed highly consistent specificity, with intense pencilline labeling of the apical membrane. In areas of superficial denudation, intermediate cells were not labeled with UPIa or UPIIIa. UPIb was localized superficially in all nine control specimens (see Fig. 1D), although two of the cases revealed additional cytoplasmic labeling of suprabasal and intermediate cells.

The two dialysis patient categories showed a very similar pattern of labeling with the uroplakin antibodies (Fig. 1, center and right columns). For UPIa and UPII, analysis of the ranked series by Kruskal-Wallis statistics revealed that there was no significant difference between the control versus the dialysis patients ($P > 0.99$ for UPIa and $P > 0.92$ for UPIIIa).

UPIb also showed two patterns of labeling: either restricted to the luminal aspect of the superficial cell (4/10 specimens) or additionally present throughout the urothelium (6/10 specimens) (Fig. 1E and F). Full-thickness UPIb expression was detected in the urothelia of three fourths "nonanuric" patients and in half of the anuric patients (3/6) ($P = 0.05$).

PPAR-γ and RXR-α expression

Nuclear PPAR-γ expression was observed in all controls and all samples revealed a differentiation-associated expression pattern with the strongest nuclear labeling in the superficial cell layer (Fig. 1M to O). In the stroma, PPAR-γ reacted with macrophages, nuclei of smooth muscle cells, and endothelial cells. Overall, the differentiation-associated expression of PPAR-γ was maintained in the two renal transplant categories. The Kruskal-Wallis test revealed no differences in the expression patterns of PPAR-γ for all three groups ($P > 0.99$).

RXR-α labeling patterns in control urothelia was similar to PPAR-γ, with a predominately diffuse labeling of the nucleus, which varied in intensity. Punctate cytoplasmic labeling was generally more pronounced than seen with PPAR-γ (Fig. 1P to R). No differences with respect to labeling patterns for RXR-α were apparent between the three groups in a blinded analysis.

CK expression

CK expression patterns were in agreement with previous reports [21]. There was no difference in the urothelial CK expression patterns between bladders with or without residual urine or the control bladders. In particular, there were no differences in the labeling patterns of basal cells. No CK14 expression was identified, supporting the absence of squamous metaplasia by histologic criteria. In all samples, CK20 expression was confined to superficial cells (Fig. 1J to L).

Ki-67 labeling indices

The Ki-67 labeling index was similar in both the anuretic (mean 0.125%, median 0.15%, range 0.04% to 0.2%) and residual urine group (mean 0.106%, median 0.1%, range 0.0% to 0.2%) and was lower than in the control group (mean 0.22%, median 0.2%, range 0.2% to 0.3%) (Table 1).

Clinical follow-up

Posttransplant, all patients experienced reasonable bladder function, defined as voiding three to seven times of normal volume of urine per 24 hours. A 5-year clinical follow-up revealed that two patients had since died of

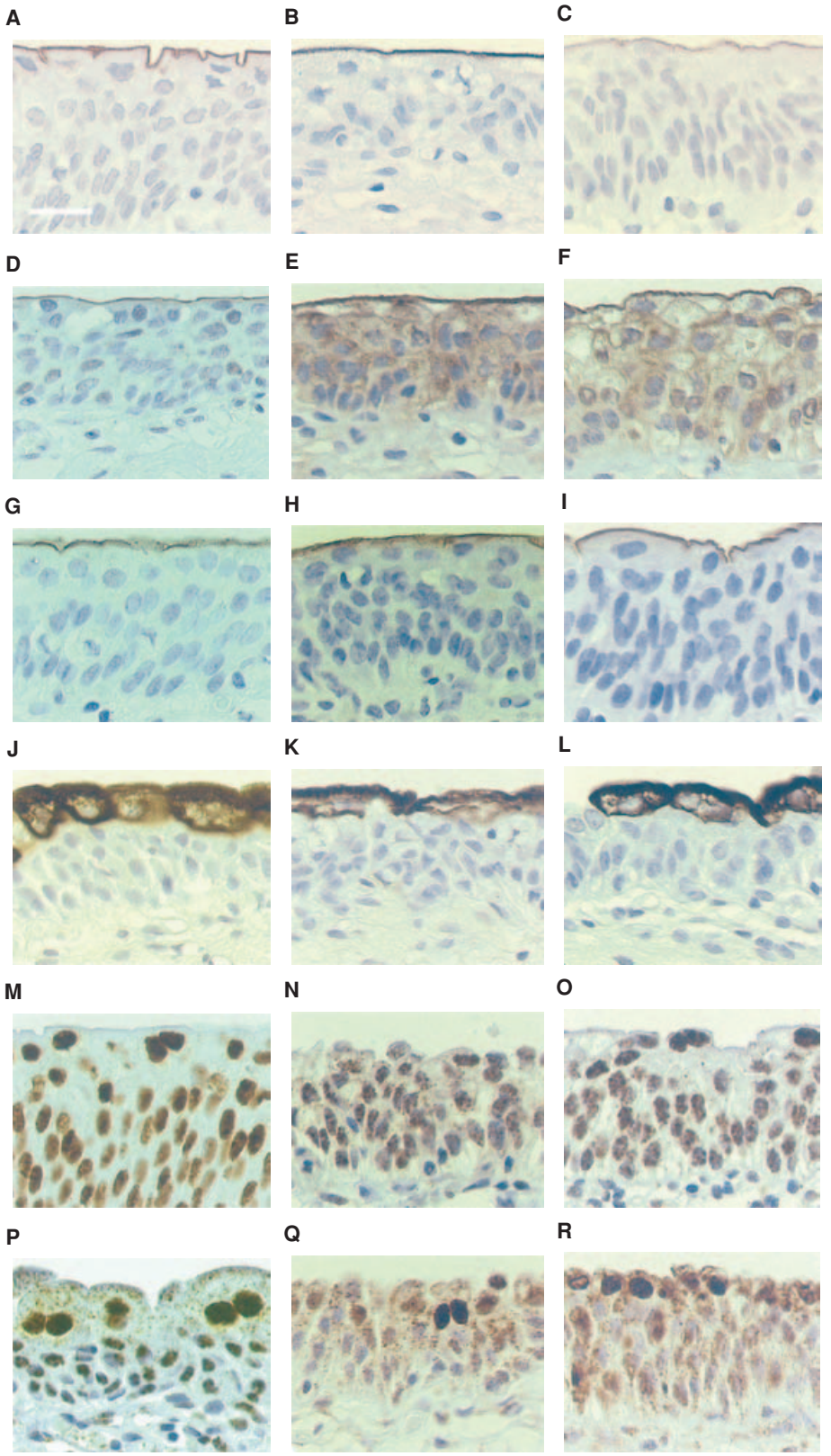

Fig. 1. Immunhistochemical expression of uroplakins UPIa (*A* to *C*), UPIb (*D* to *F*), UPIIIa (*G* to *I*), CK20 (*J* to *L*), peroxisome proliferator-activated receptor-γ (PPAR-γ) (*M* to *O*), and retinoid X receptor-α (RXR-α) (*P* to *R*) in controls (left column), "non-anuric" (middle column), and "anuric" (right column) urothelium. All sections counterstained with hematoxylin [original magnification ×600; bar in (A) 100 μm]. Note the preservation of the labeling patterns for UPIa (A to C), UPIIIa (G to I), CK20 (J to L, PPAR-γ (M to O), and RXR-α (P to R) in all three groups. UPIb (D to F) revealed the greatest variation in expression, which was also noted in control specimens. Note persistent decoration of the superficial membrane region even in the case of cytoplasmic UPIb expression (E and F).

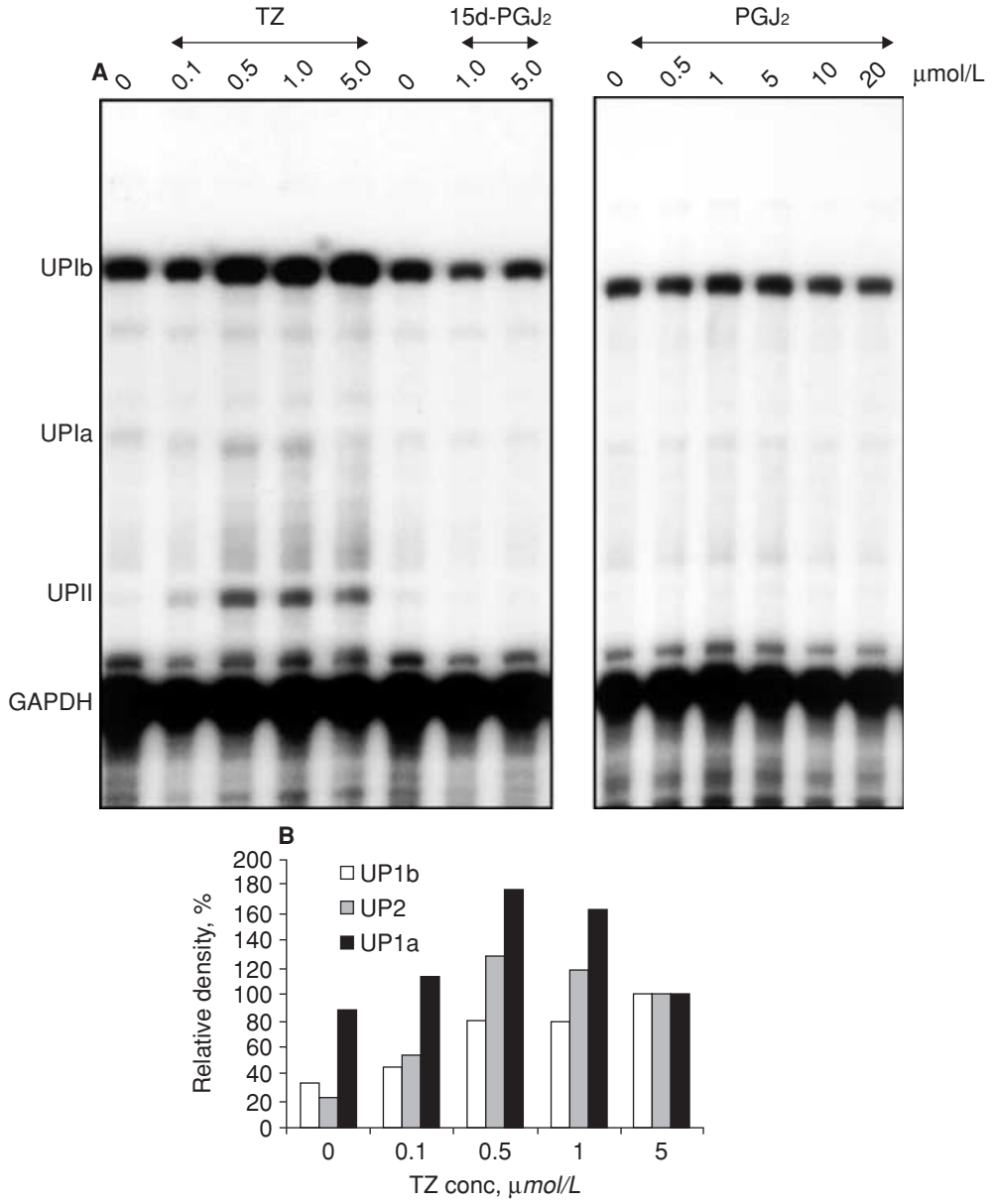

Fig. 2. Effect of peroxisome proliferator-activated receptor (PPAR) ligands on uroplakins (UP) mRNA expression. (*A*) Normal human urothelial (NHU) cells were pretreated for 24 hours in the absence or presence of troglitazone (TZ), 15-deoxy-Δ12,14-prostagladin J_2 (15d-PGJ_2), and prostaglandin J_2 (PGJ_2) at the concentrations indicated. Next, the cells were incubated for 4 days in medium treated with the epidermal growth factor (EGF) receptor inhibitor, PD153035 (1 μmol/L). Medium was changed with "fresh" PD153035 added every 2 days. Total RNA was extracted and 5 μg was hybridized with ^{32}P-labeled human uroplakin UPIa, UPIb, UPII, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probes. The samples were electrophoresed on a 5% polyacrylamide gel. (*B*) The uroplakin bands were quantified by means of a phosphorimager and normalized against the GAPDH which was used to standardize for loading efficiency. Troglitazone at 5 μmol/L concentration was taken to be 100%.

causes unrelated to bladder-renal function. The remainder of the patients reported no symptoms regarding their bladder function.

Effect of 15d-PGJ_2, PGJ_2, and troglitazone on expression of uroplakin mRNA

NHU cells did not express UPIa, UPII, or UPIIIa under normal culture conditions, although there was constitutive expression of UPIb mRNA, in agreement with previous reports [7, 20]. Previously, we have demonstrated that the PPAR-γ ligand, troglitazone, induces the expression of UPIa and UPII, when EGF signaling is blocked [7]. Treatment of EGFR-inhibited NHU cells with 15d-PGJ_2 or PGJ_2 did not result in any increase in expression of mRNA for any of the uroplakins (Fig. 2A). As a positive control, when EGFR-inhibited NHU cells were

treated with troglitazone, there was a dose-dependent induction of UPIa, UPIb, and UPII mRNA expression at concentrations of troglitazone of up to 1 μmol/L (Fig. 2B).

DISCUSSION

PPAR-γ signaling has been implicated in the differentiation of adipocytes [22], carcinoma cell lines [23–25] and more recently, NHU cells [6, 7]. In the latter case, pharmacologic activation of PPAR-γ in cultures of NHU cells led to transcription of genes associated with terminal urothelial cytodifferentiation and raises the interesting question of how this relates to the in vivo situation. Given the superficial accentuation of PPAR-γ expression and the presence in urine of the putative PPAR-γ agonist, 15d-PGJ_2, it was considered that 15d-PGJ_2 may act through PPAR-γ to induce urothelial differentiation in superficially-positioned cells. Such a hypothesis is compelling, as it would provide a mechanism for differentiation and the development of urinary barrier function in intermediate urothelial cells exposed to urine following exfoliation of superficial cells.

Following the establishment of a predominately differentiation-associated pattern for the expression of PPAR-γ and its heterodimerization partner RXR-α in normal urothelium [6, 7], the rationale of this study was to determine whether lack of exposure to normal urine affected proliferation or the expression of urothelial differentiation-associated markers. For this purpose, the urothelium from patients on maintenance hemodialysis for ESRD, with or without residual renal function, was investigated. Our results suggest that urinary factors are inconsequential to the maintenance of urothelial cytodifferentiation. Furthermore, as such bladders lack physiologic filling and voiding cycles, cyclic mechanical changes also appear unnecessary to maintain a differentiated urothelium. Although the absolute number of specimens studied was small, they came from a rigorously selected group of patients with end-stage renal failure and on dialysis for more than 2 years. We believe that the lack of evidence for a change in the differentiation status of the urothelium provides prima facie evidence that bladder function is not involved in maintaining urothelial differentiation.

In humans, uroplakins UPIa, UPII, and UPIIIa are highly urothelium- and differentiation-specific [26] and all revealed highly restricted immunolocalization to the apical aspect of superficial cells. Only UPIb displayed a degree of variable expression, showing an apical localization consistent with its role in the asymmetric unit membrane plaque, but with additional diffuse labeling throughout the urothelium in some specimens. The finding that UPIb showed the least differentiation-specific profile is consistent with other data; the uroplakins exist as heterodimers in the asymmetric unit membrane plaques and this state is obligate for all but UPIb, which is therefore the only uroplakin to exist outside of the asymmetric unit membrane plaque [2, 27]. Furthermore, of all the uroplakin genes, UPIb is the least tissue- and differentiation-restricted [26, 28] and is expressed by nondifferentiated NHU cell cultures and by 50% superficial urothelial cell carcinomas [20]. It is unknown why UPIb is less differentiation-restricted than the other uroplakins, but it is possible that it may have alternative, nonasymmetric unit membrane–related function(s), which have yet to be elucidated. Nevertheless, there was no statistical difference in UPIb expression pattern that related to whether the urothelium had been exposed to normal urine or not and a similar "broadening" of expression was found in some normal control specimens. This implies that changes in UPIb expression did not relate directly to the deprivation of urine or other bladder function.

The identification of 15d-PGJ_2 as the final metabolite of the PGJ_2 series (reviewed [29]) led to its recognition as a candidate natural ligand of PPAR-γ [30, 31], which is present in human urine [32]. Men excrete approximately 152 ng/24 hours of urinary 15d-PGJ_2, almost double the amount excreted by females [14], although these gender-related findings are not consistently reported [32]. Interestingly, the present study demonstrated that bladder urothelium from patients on maintenance hemodialysis for ESRD revealed a terminally differentiated urothelial phenotype, independent of the duration of dialysis (maximum dialysis time 79 months), or the presence or absence of intravesical residual urine due to residual renal function.

The study also demonstrated that the long-term absence of urinary factors had no effect on the quiescent nature of urothelium. Anuric patients and patients with residual renal function had on average the same proportion of cells in the mitotic cell cycle (0.1%), which was half that of the control group (average 0.2%).

The maintenance of a differentiation-associated expression pattern of PPAR-γ and RXR-α in urothelium, in the absence of urine, does not support the hypothesis that urine-derived factors, including PGJ_2 and 15d-PGJ_2, are necessary factors in the maintenance of a differentiated urothelial phenotype. In vitro studies have generally used 15d-PGJ_2 at concentrations in the micromolar range. The median effective concentration (EC_{50}) for 15d-PGJ_2 to promote efficient differentiation of C3H10T1/2 fibroblasts to adipocytes was 7 μmol/L [30] and the effective concentration of 15d-PGJ_2 for activation of PPAR-γ in gene reporter studies was 3 μmol/L [31]. Three micromoles per liter was also the effective growth inhibitory concentration of 15d-PGJ_2 for NHU cells in vitro [11]. Nevertheless, even this concentration of 15d-PGJ_2 would be 5000 times higher than the presumed in vivo concentration of 15d-PGJ_2 in the urine of men, suggesting that the concentrations in urine are biologically

ineffective. Although prostaglandin transporters (PGT) have recently been identified in man, *Xenopus* oocyte transfection models for prostaglandin E_2 (PGE_2) were only able to produce a maximum 15-fold concentration gradient [33, 34]. Moreover, there is no PGJ_2 transporter gene expression reported in urothelium [34]. The role of 15d-PGJ_2 as a physiologic ligand for PPAR-γ is the subject of some controversy [34] and our results support the view that it is not a strong candidate.

Nevertheless, the strong differentiation-associated expression of PPAR-γ is highly suggestive of an involvement in urothelial differentiation. One possible explanation for the maintenance or expression of PPAR-γ in urothelium is that a PPAR-γ–specific ligand is derived from the urothelial cells themselves in an autocrine fashion, since PPAR-γ expression is self-regulated [34]. Jowsey et al [35] determined gene expression of PPAR-γ and of prostaglandin D_2 synthase (PGDS), the key enzyme producing prostaglandins of the D and J series, and demonstrated coexpression in a number of human tissues and cell types, including adipose tissue, placenta, prostate, and macrophages. Unfortunately, urothelium was not included in their study. However, these data reveal the potential for de novo prostaglandin synthesis in the context of PPAR-γ expression in human epithelial cells, and suggest that the endogenous production of fatty acid metabolies, such as prostaglandins, may contribute to PPAR-γ signaling in vivo.

CONCLUSION

This is the first study to systematically investigate bladder urothelium from patients on maintenance hemodialysis with normal bladder function and no history of urinary symptoms. Studies assessing the urothelium of patients with normal bladder function on maintenance dialysis are sparse and have primarily focused on the development of benign or malignant neoplastic lesions [36, 37]. Our results imply that urothelium is able to maintain its highly differentiated phenotype and function over a long period of time even in the absence of its usual environmental interphase of urine or mechanotransduction and suggest that other (intrinsic or extrinsic) factors must be involved in regulating the differentiated phenotype.

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